Application No.: 10/620,806

## Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

## **Listing of Claims:**

1. (Currently Amended) An assay for determining level of amount of prostacyclin in a plasma sample comprising:

- (1) providing a plasma sample on a surface coated with an anti-immunoglobulin antibody;
- (2) incubating the plasma sample with an effective amount of an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF<sub>1 $\alpha$ </sub>) antibody[[;]] wherein the [[an]] anti-immunoglobulin antibody that-binds to the anti-6-keto-PGF<sub>1 $\alpha$ </sub>-antibody; and a conjugate comprising 6-keto-PGF<sub>1 $\alpha$ </sub> covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74(Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (3) removing any unbound anti-6-keto-PGF<sub>1 $\alpha$ </sub>-antibody and said conjugate from the plasma-sample following incubation; and
- (4) measuring and correlating light intensity of the  $\underline{6\text{-keto-PGF}_{1\alpha}}$  bound to the antiimmunoglobulin antibody plasma sample; and with amount of prostacyclin within the plasma sample
- (5) correlating the light intensity of the <u>bound</u> 6-keto- $\underline{PGF_{1\alpha}}$  plasma sample, with the amount of prostacyclin in the plasma sample.
  - 2. (Cancelled)
  - 3. (Cancelled).
- 4. (Original) The assay of claim 1 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

**Application No.: 10/620,806** 

- 5. (Currently Amended) The assay of claim 1 wherein the concentration of said conjugate in the assay has a concentration of [[is]] about  $1 \times 10^{-10}$  M.
  - 6. (Cancelled).
  - 7. (Cancelled).
- 8. (Currently Amended) A method of determining an appropriate dose of amount of prostaglandin in a plasma sample for the treatment of primary pulmonary hypertension in a patient-comprising:
- (1) providing a plasma sample from the patient <u>on a surface coated with an anti-</u>immunoglobulin antibody;
- (2) incubating the plasma sample with an effective amount of an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF<sub>1\alpha</sub>) antibody[[;]] wherein the [[an]] anti-immunoglobulin antibody that-binds to the anti-6-keto-PGF<sub>1\alpha</sub>-antibody; and a conjugate comprising 6-keto-PGF<sub>1\alpha</sub> covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74(Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and

wherein the 6-keto-PGF $_{1\alpha}$  is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine,

- (3) removing any unbound anti-6-keto-PGF<sub>1 $\alpha$ </sub>-antibody and said conjugate from the plasma sample following incubation; and
- (4) measuring and correlating light intensity of the amount of detected 6-keto-  $PGF_{1\alpha}$  bound to the anti-immunoglobulin antibody; and
- (5) correlating the light intensity of the bound 6-keto-  $PGF_{1\alpha}$  with the appropriate dosage of prostaglandin for the patient amount of prostaglandin in the plasma sample.
  - 9. (Cancelled).

## Application No.: 10/620,806

- 10. (Cancelled).
- 11. (Currently Amended) The assay method of claim 8 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.
- 12. (Currently Amended) The assay method of claim 8 wherein the concentration of said conjugate in the assay is about  $1 \times 10^{-10}$  M.
  - 13-21. (Cancelled)
  - 22. (Currently Amended) A kit for measuring prostacyclin in plasma comprising:
  - (1) an anti-6-keto-prostaglandin  $F_{1\alpha}$  (6-keto-PGF<sub>1\alpha</sub>) antibody;
  - (2) an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and
- (3) a conjugate comprising 6-keto-PGF<sub>1 $\alpha$ </sub> covalently bound to an aequorin mutant; wherein said aequorin mutant comprises serine substitutions substitutions for all three cysteine residues as present in wild-type aequorin (Cys  $\rightarrow$  Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69  $\rightarrow$  Cys), 70 (Gly70  $\rightarrow$  Cys), 74(Gly74  $\rightarrow$  Cys) or 76 (Glu76  $\rightarrow$  Cys), and wherein the 6-keto-PGF<sub>1 $\alpha$ </sub> is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine.